Immune Response

With Particular Reference to the Use of Multiple Antigens

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■ *The increasing demand for preventive child health services and the general increase in international travel compel greater attention to the use of multiple antigens, both inactivated and live, when administered simultaneously. It appears that with the preparations currently licensed, multiple inactivated antigens may be given safely and with expectation of optimal effectiveness.* DPT *is a routine combination employed in combination with oral trivalent poliovaccine for primary immunization of infants and young children up to and including age six. Oral poliovirus vaccine and vaccinia may be administered at the time of the recall or booster dose of* DPT *vaccine during the second year of life, commonly at age 15 to 18 months.*

It is apparent from published data accumulated over many years that several antigens may be administered at the same time with adequate immunologic response. The minor differences in antibody response following simultaneous administration of live viral antigens is of unknown clinical importance. The primary reason for hesitancy in advocating greater use of multiple agents at this time is the theoretical consideration of possible neurotoxicity with those vaccines where the parent agent may have definite neurotoxicity. The question of possible additive or other harmful effects with measles, poliomyelitis, and rubella and mumps when given simultaneously can be answered only by carefully controlled studies involving close observation of the recipients with extension of these trials as data permit.

THE AVAILABILITY of several relatively new vaccines has directed attention toward developing improved schedules for immunization. The goal is to provide maximal host response with minimal reaction, and at the same time not unnecessarily complicate the immunization process for either the patient or the physician.

The need to provide protection routinely in this country against the eight diseases noted in Table 1 for which antigens are at present available has been amply demonstrated. The extension of com-

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TABLE 1.—*Antigens Currently Available for Immunization*

FOR USE IN THE UNITED STATES	
INACTIVATED ANTIGENS	ATTENUATED VIRAL ANTIGENS
Diphtheria	Measles
Pertussis	Mumps
Tetanus	Poliomyelitis (OPV)
Poliomyelitis (IVP)	(Rubella)*
Influenza	
FOR OVERSEAS TRAVEL	
INACTIVATED ANTIGENS	ATTENUATED VIRAL ANTIGENS
Cholera	Yellow Fever
Typhoid	
Typhus	

*Licensure expected in 1969 or 1970.

prehensive health services employing these antigens to population groups poorly immunized in the past has presented problems, since appointments may be broken frequently, often for compelling reasons. For such persons the temptation is great to provide a maximum number of immunizations when the patient is in the office or clinic. The foreign traveler with additional requirements frequently appears in the office or clinic for the first time only a few days before departure, also complicating the usual planned sequence of immunizations. In addition to these problems, the newer techniques of purification of antigens will undoubtedly lead to the prospect of many highly specific antigens which theoretically could be included in a single injection of small volume.

To develop an understanding of some of the problems involved and the measures to insure adequate response, we must first review the characteristics of the immune response of the host and the factors which may influence this response which are inherent in the use of multiple antigens in the clinical situation.

Response to Inactivated Antigens

Following injection, antigen may be demonstrated within the cytoplasm of lymphocytes, macrophages, primitive reticulum cells and immature plasma cells. Of these, the plasma cell appears to be most important in the production of antibody. After a latent or induction phase lasting from one to several days the production of antibody begins, and the plasma cell appears most important in its production. During this productive phase, antibody levels increase and reach a point which is dependent upon the characteristics of the antigen administered, the route, the dose (whether in a single site or multiple simultaneous initial injections), and whether an adjuvant such as alum or aluminum hydroxide has been included.[1]

Although the site and mechanism of antibody production has been amply documented,[2,3] there has been considerable doubt as to whether individual antigens have separate "populations" of lymphocytes and plasma cells serving for immunologic memory and antibody production respectively, or whether a single population of cells within the host may respond to several antigens simultaneously. Recent data suggest that a single cell is capable of producing at least two separate antigens simultaneously.[4] In the test situation described by Michael and Marcus it was apparent that two antigens provided simultaneous stimulation of a large proportion of single individual cells to produce two separate and distinct antibodies, although the actual amount of each antibody produced was less than that when the cell was engaged in producing a single type in response to a single antigen.

Effect of Multiple Antigens Given Simultaneously

More than 50 years ago it was found that experimental animals could respond to more than a single antigen simultaneously. These early data suggested that the host response in the intact animal was sometimes less when multiple antigens were given in combination than when each antigen was given individually. Studies including up to 35 antigens administered simultaneously have indicated that the response to a single agent may be adequate in a combination, although a "crowding out" effect may be seen when an excessive concentration of a single antigen is included in the mixture. In this latter situation it would appear that the individual devotes an excessive amount of energy toward production of antibody in response to the antigen in excessive dosage to the detriment of response to others in the mixture. This phenomenon has been shown particularly clearly when the test animals had previously received a primary dose of one of the antigens included in the mixture. The secondary response to this antigen was associated with decreased response to the other antigens.[5,6,7] Further studies of this "crowding out" or interfering phenomenon among inactivated antigens have suggested that adjusting the amount of antigen administered and beginning immunization simultaneously with several antigens in proportional amounts avoids clinically important interference. Indeed, the adjuvant effect of pertussis antigen when included with diphtheria and pertussis has

been noted to enhance the response to diphtheria antigen with this combination.[8] However, it should be noted that with large quantities of pertussis antigen, even in this mixture, it is possible to depress the response to tetanus and diphtheria toxoid.[9]

It would appear that, using the currently available inactivated antigens, it is possible to give pertussis, diphtheria, tetanus and the three polioviruses simultaneously without untoward effect and with at least the customary response.[10,11] However, these mixtures may lack stability after prolonged storage and are not at present recommended for use. With the mixture of diphtheria, tetanus and pertussis currently available, it would appear that the toxoid response may be enhanced somewhat by the incorporation of pertussis vaccine.[12,13] Insufficient data are available concerning simultaneous administration of adult type diphtheria, tetanus, cholera, typhoid, and typhus vaccines to permit firm conclusions. The immunologic response following simultaneous administration of tetanus, diphtheria, typhoid, paratyphoid A and B and three inactivated poliovirus antigens was adequate for all except for a relatively poor response to the typhoid and paratyphoid A and B components,[14] this deficiency perhaps reflecting earlier immunization with other vaccine components.

Although only of historic interest currently, it should be noted that the three poliovirus antigens and inactivated measles vaccines, when administered simultaneously, produced serologic responses comparable to the responses evoked by these antigens when used individually.[15,16]

Spacing of Multiple Antigens

As noted above, if the individual has had previous experience with one of the antigens represented in a combination of inactivated antigens depression in expected response may occur with those antigens administered for the first time. Until more information is available, it would be desirable to administer at least some inactivated antigens simultaneously. An example of an inappropriate schedule might be the use of an initial dose of pertussis antigen given alone in an infant and then following it with diphtheria, tetanus and pertussis in combination. The preferable course would be to use DPT simultaneously. For the physician dealing with adults, it may be preferable to administer cholera and typhus vaccine to the international traveler in advance of adult type diphtheria-tetanus toxoid, since the latter is more likely to result in a response of recall type due to previous experience with the antigens. However, it should be pointed out that these considerations may be largely theoretical and should not prevent the simultaneous use of these antigens should the traveler be departing for his destination within a short time and require at least some protection before departure.

Immunologic responsiveness is greater if the doses of vaccine administered during the primary immunization series are given at intervals separated by more than a few days or weeks. Dr. Jeannette Wilkins, in recent studies with pertussis vaccine, showed that two doses of the currently standardized vaccine given at 60-day or greater intervals to infants 2 months of age or older provide the same agglutinin responses as three doses given at 30-day intervals. If this holds true with other antigens, and if the response seen is as durable as that following larger numbers of doses, this may result in further simplification of schedules. Both in experimental animals and in man extension of the interval between doses in the primary series is likely to result in at least as great a response if the doses are separated by several weeks or months than if the doses are given at one-month intervals. By wider spacing of doses a secondary or booster effect is often seen, and the practical advantage of a response of this type is obvious. The often observed but wasteful practice of beginning schedules over again when patients fail their appointments should be avoided.

Age of Initial Immunization

The blanketing effect of passively acquired maternal antibody in newborn or young infants has been amply demonstrated against a number of inactivated antigens, given singly or in combination.[17] This blanketing effect, although clearly depressing the initial response to several antigens, has no apparent effect upon reinforcing or booster doses given during subsequent months or years. Therefore, with inactivated antigens this factor appears to be clinically unimportant.

It should also be noted that the immune response of the newborn infant appears to be largely an IgM response, and that this characteristic response persists during the first few weeks of life. Furthermore, the ultimate antibody titers achieved are less than those of older infants or adults. This immunologic immaturity appears to be associated with a scarcity of plasma cells in tissues respon-

sible for antibody formation, and this deficiency may be the factor responsible for the relatively ineffective response. Indeed, it is important to note that the only clinical demonstration to date of possible immunologic paralysis in man was that of Provenzano, Wetterow, and Sullivan[18] who suggested that immunologic tolerance against pertussis vaccine may have occurred in some infants receiving large numbers of doses (three to six) beginning within 24 hours of birth and continued at three-week intervals. Although immunologic paralysis in experimental animals was described in 1949 by Felton, it has not been believed important in man since excessive doses of antigen were required to induce this phenomenon in animals and the intravenous route appeared to be essential for most animals. Although it was originally thought that antibodies were being formed during immunologic paralysis, but were not demonstrable since they were bound so promptly by excess circulating antigen, it now appears that these concepts may be incorrect. There now is clear evidence for lack of any antibody production during immunologic paralysis[19] and it appears that this is at least a theoretical possibility in man with large doses of antigen administered at an early age.

Untoward Effects Following Inactivated Vaccines

Reactions of consequence following the use of currently available inactivated antigens are almost unknown. It is important to note, however, that recently the administration of inactivated measles vaccine has been followed by untoward local and systemic reactions with subsequent attenuated measles vaccine immunization[20] or with naturally acquired infection. These reactions suggest that inactivated measles vaccine should no longer be used. Use of an experimental inactivated respiratory syncytial vaccine has been followed by increased frequency of symptomatic disease in infants subsequently encountering the natural infection.[21] These observations suggest new facets of considerable importance in the prophylaxis of disease, and may lead to a better understanding of the role of IgA secretory antibody in prevention of disease as well as of IgG in the pathogenesis of some diseases.

In summary, it would appear that the use of several inactivated antigens is on a sound foundation, providing excessive concentrations of quantities of a single component in the mixture are not employed. It would further seem desirable to avoid administering a mixture involving antigens to which the host has not had previous exposure in combination with one or more in which a primary sensitizing dose had been given months or years previously. Even in this latter situation, some response may be expected to each of the individual components in the mixture, although this response may not necessarily be comparable to that seen when that component is given individually.

Attenuated Viral Vaccines

Considerations with regard to the simultaneous administration of live attenuated viral antigens are quite different from those outlined above for the inactivated antigens. With the administration of live attenuated antigens, factors such as the presence of circulating passively acquired antibody against that agent, interference between viruses if more than a single live virus is administered at one time, and the theoretical consideration of the possible selection of more virulent particles during the replication of the vaccine strain in the host must be considered. Of these, the easiest to deal with on the basis of current data is the effect of circulating antibody upon efficacy of single or multiple live virus antigens.

The prompt neutralization of live or active viral vaccines may have a profound effect upon immunologic response to these agents during much of the first year of life. This inhibition of response has been clearly demonstrated by many investigators using live measles and mumps vaccine, and preliminary data are available concerning inhibition of rubella vaccine at ages less than one year as well. With respect to vaccinia, adequate host response has been seen in infants less than one year of age, although data clearly suggest that complication rates are somewhat higher during the first year of life than in infants immunized after they are a year old.[22]

Consequently, it would appear undesirable to use presently available attenuated viral vaccines (measles and smallpox) at less than one year of age, with the obvious exception of the oral poliovirus strains. The latter appear to be safe and effective when administered to infants during the first few months of life. Immunologic response has been satisfactory and the period of infancy is a time when protection against poliomyelitis is most important.

Interference between live viruses was first dem-

onstrated in 1935 by Magrassi and by Hoskins. The discovery of interferon by Isaacs and Lindeman in 1957 indicated at least one possible mechanism for the observed viral interference. It is important to note that the vaccine strains of several of the currently licensed vaccines are particularly efficient, when compared with the wild viruses of the same type, in producing interferon in both man[23,24] and tissue culture systems.[25] Despite this phenomenon, numerous investigations of poliomyelitis Types I, II and III when given simultaneously; combinations of poliovirus and attenuated measles virus;[26] poliomyelitis and smallpox;[27] vaccinia and yellow fever,[28,29] measles and vaccinia;[30-33] measles, smallpox, and yellow fever;[34] and measles and mumps[35] have indicated that combinations of various live viruses have been used with a relatively high degree of effectiveness as measured by circulating antibody response. With the several combinations noted, the only discernible effect has been a slight depression in the level of circulating antibody against one individual component of the mixture, most frequently with yellow fever or a depression in subsequent antibody titer but not in frequency of "takes," with vaccinia. The clinical importance of such relatively minor depressions of host response is not clear at this time.

It is of interest that simultaneous use of vaccinia and BCG vaccine has been followed by some increase in pustular reaction to BCG.[36]

Some vaccine strains, notably polioviruses Types I, II and III, do not replicate as efficiently in the laboratory at elevated temperatures comparable to febrile episodes in man as do the wild or naturally occurring strains. It has been postulated by some investigators that administration of other antigens which may induce a febrile reaction may selectively favor a more virulent vaccine population by favoring an occasional virus particle of greater virulence. Whether this is of any importance, or whether, even if possible, the interferon response to vaccine strains may negate such an effect remains to be seen. Certainly the experimental data with oral poliovaccines and other attenuated viruses cited above, and the widespread use of oral poliovaccines with DPT vaccine,[37-39] together with frequent unexplained febrile episodes among young infants, provide reassurance that this possibility is of little or no importance. Concern over possible additive effects of simultaneous administration of vaccines whose wild parent strains occasionally produce encephalitis can be answered only by further experience. Examples of these might be mumps, measles and rubella. The data available at present are not sufficient to answer this question.

Further exploration of the simultaneous use of multiple live viral vaccines is urgently needed. At present, on the basis of both experimental data and widespread usage, poliomyelitis vaccine together with DPT or vaccinia or both may be administered simultaneously without fear of untoward reaction or complications. Data at present suggest that many other combinations of attenuated viruses may be employed in the future and their use must be explored in carefully observed populations with adequate laboratory controls.

Current recommendations for the use of multiple live viral antigens have been developed by the Public Health Service Committee on Immunization Practices. These recommendations are as follows:

Simultaneous Administration of Live Virus Vaccines:

> Data on simultaneous administration of live virus vaccines are not sufficient to develop comprehensive recommendations; but there are obvious practical advantages to combining vaccines, and investigations are under way which should help to define optimal practices. When combined administration is indicated, available data do not suggest that undesirable responses will result. The following comment presents current attitudes toward scheduling vaccination with three major live virus vaccines — poliomyelitis, measles and smallpox.
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> It has been generally recommended that immunizations with live virus vaccines be separated by at least one month whenever possible. The rationale for this recommendation is the theory that superimposed reactions and diminished antibody responses might result if two or more live virus vaccines were given simultaneously. Ideally, the initial doses of oral poliovirus vaccine should have been given before a child reaches one year, the age for giving live attenuated measles virus vaccine. Administration of poliomyelitis and measles antigens should be separated by at least one month. It is likewise desirable to separate measles and smallpox vaccinations by one or more months because both of these antigens may produce febrile reactions.

When, however, immunization program effectiveness is hindered or when the threat of concurrent exposures exists, the relevant live virus vaccines should be given at the same time. Observations do not indicate that this will cause a significant increase in adverse reactions or depressed antibody responses to either antigen.

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